Selective Immunosuppression by Administration of Major Histocompatibility Complex (MHC) Class II-binding Peptides. I. Evidence for In Vivo MHC Blockade Preventing T Cell Activation

By Jean-Charles Guéry,* Alessandro Sette,‡ Jonathan Leighton,* Alin Dragomir,* and Luciano Adorini*

*From the *Preclinical Research, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland; and ‡Cytel Corp., San Diego, California 92121*

Summary

Draining lymph node cells (LNC) from mice immunized with hen egg white lysozyme (HEL) display at their surface antigen-MHC complexes able to stimulate, in the absence of any further antigen addition, HEL peptide-specific, class II-restricted T cell hybridomas. Chloroquine addition to these LNC cultures fails to inhibit antigen presentation, indicating that antigenic complexes of class II molecules and HEL peptides are formed in vivo. MHC class II restriction of antigen presentation by LNC from HEL-primed mice was verified by the use of anti-class II monoclonal antibodies. Coinjection of HEL and the I-A^k-binding peptide HEL 112–129 in mice of H-2^k haplotype inhibits the ability of LNC to stimulate I-A^k-restricted, HEL 46–61-specific T cell hybridomas. Similar results are obtained in mice coinjected with the HEL peptides 46–61 and 112–129. Inhibition of T hybridoma activation can also be observed using as antigen-presenting cells irradiated, T cell-depleted LNC from mice coinjected with HEL 46–61 and HEL 112–129, ruling out the possible role of either specific or nonspecific suppressor T cells. Inhibition of T cell proliferation is associated with MHC-specific inhibition of antigen presentation and with occupancy by the competitor of class II binding sites, as measured by activation of peptide-specific T cell hybridomas. These results demonstrate that administration of MHC class II binding peptide competitors selectively inhibits antigen presentation to class II-restricted T cells, indicating competitive blockade of class II molecules in vivo.

Peptides bound to class II MHC molecules on the surface of APC are the ligand for specific TCRs of $CD4^+$ T cells (1). In the past few years, peptide-MHC interactions have been characterized in detail by structural, biochemical, and functional analysis (reviewed in reference 2), clarifying at the molecular level several aspects of the binding of antigenic peptides to class I and class II MHC molecules. Since each MHC class II molecule binds many different peptides, peptides of rather different sequences can compete for antigen presentation by a given class II molecule to T lymphocytes.

Peptide competition for antigen presentation has been demonstrated in vitro, by showing that peptides binding to the same class II molecule can compete with each other for presentation to T cells (3–6). Several studies have clearly demonstrated a direct correlation between the capacity of a peptide to bind to purified class II molecules and its ability to compete for presentation with antigenic peptides binding to the same class II molecule (7, 8).

It has been postulated that peptide competition for the MHC class II binding site may occur also in vivo (9). This was first implied by the observation that self-peptides could compete with non-self antigens for T cell priming (10). In addition, in vivo competition between different peptides derived by processing of the same protein antigen has been shown to profoundly influence the immunodominance of T cell determinants (11).

More recently, several groups (12–15) have reported that this approach could be applied to prevention of experimental autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE)[1] or autoimmune carditis. However, in most of the EAE studies (12, 13), disease induction by encephalitogenic peptides was prevented by coinjection of a close analogue of the encephalitogenic peptides, raising the

[1] *Abbreviations used in this paper:* EAE, experimental autoimmune encephalomyelitis; HEL, hen egg-white lysozyme; LNC, lymph node cells.

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possibility of antigen-specific, rather than MHC-specific, mechanisms in disease prevention (16). Indeed, this was later demonstrated to be the case (17). EAE has also been prevented by injection of a competitor peptide nonhomologous to the encephalitogenic peptide, but since the competitor peptide was itself immunogenic, clonal dominance in the T cell response to the competitor could not be excluded as a possible cause of EAE prevention (14).

To clarify these issues and to examine more directly the mechanism inhibiting in vivo T cell activation by administration of class II–binding peptide competitors, we first established an ex vivo system to detect complexes between antigenic peptides and class II molecules generated in vivo and expressed on the surface of lymph node cells (LNC). Then, we coinjected antigenic and competitor peptides of different sequences that bind to the same class II molecule, and examined the in vivo formation of antigenic peptide-MHC complexes. Results in the present paper demonstrate that, under these conditions, administration of class II–binding peptide competitors specifically inhibits in vivo the capacity of LNC to present antigen to MHC class II–restricted T cells.

Materials and Methods

Mice. 8-wk-old C3H, B10.D2, and DBA/2 female mice, isolator reared and virus free, were obtained from Iffa Credo (L' Arbesle, France).

Antigens. HEL (recrystallized three times) was obtained from Sigma Chemical Co. (St. Louis, MO). Peptides were synthesized by the solid-phase method on PAM-polystyrene support using side-chain protection, coupling procedures, and an automated apparatus (430A; Applied Biosystems, Inc., Foster City, CA), as described (10). The peptides were purified by preparative HPLC on a C18 reverse-phase column and showed correct amino acid ratios upon hydrolysis in 6 N HCl and the expected molecular ions in fast atom bombardment mass spectrometry. Sequences were confirmed by gas-phase microsequencing.

T Cell Proliferation. Mice were immunized subcutaneously into the hind footpads with 1–14 nmol antigen (HEL or HEL peptides) emulsified in CFA containing H37Ra mycobacteria (Difco Laboratories, Detroit, MI). 8 d later, popliteal lymph node cells were cultured (4×10^5/well) in microtiter plates (Costar, Cambridge, MA) in HL-1 medium (Ventrex Laboratories, Portland, ME). Cultures were set up in triplicate from pooled LNC of individual mice. Cultures were incubated for 3 d in a humidified atmosphere of 5% CO_2 in air and pulsed 10 h before harvesting with 1 μCi [^{3}H]TdR (40 Ci/mmol; The Radiochemical Center, Amersham, UK). Incorporation of [^{3}H]TdR was measured by liquid scintillation spectrometry.

T Cell Hybridomas. T cell hybridomas were established by polyethylene glycol–induced fusion of LNC with the thymoma line BW5147, as previously described (8, 11). LNC were obtained from mice immunized with HEL-CFA and restimulated in vitro with 7 μM HEL before cell fusion. Cultures containing 5×10^4 T hybridoma cells and 2.5×10^4 LK-35.2 ($H\text{-}2^{k/d}$) cells (obtained from the American Type Culture Collection, Rockville, MD) were set up with or without antigen in 0.2 ml of culture medium. Culture medium was RPMI 1640 (Gibco, Basel, Switzerland) supplemented with 2 mM L-glutamine, 50 mM 2-ME, 50 μg/ml gentamicin (Sigma Chemical Co.) and 10% FCS (Gibco). After 24 h of culture, 50-μl aliquots of supernatant were transferred to microculture wells containing 10^4 CTLL cells and, after an additional 24-h incubation, the presence of T cell growth factors (TCGF), mainly IL-2, was assessed by [^{3}H]TdR incorporation during the last 4 h of culture. Hybridoma cells reactive to HEL peptides were cloned by limiting dilution at 0.3 cells/well. The MHC class II molecule involved in antigen recognition by individual T cell hybridomas obtained from C3H mice was determined by comparing the TCGF production obtained in the presence of irradiated (2,400 rad) spleen cells from C3H (expressing $I\text{-}A^k$ and $I\text{-}E^k$ molecules) and B10.A(4R) (expressing only $I\text{-}A^k$ molecules) mice. The $I\text{-}E^d$-restricted (8) and the $I\text{-}E^k$-restricted (18) T cell hybridomas have been previously described. The 3A9 T hybridoma (19) was a kind gift of Dr. Paul Allen (Washington University, St. Louis, MO). The T cell hybridomas used in the present study are listed in Table 1.

T Cell Activation Assays. HEL-specific T cell hybridomas (5×10^4 cells/well) were cultured in duplicate or triplicate with a dose range ($0.1\text{–}1 \times 10^6$) of irradiated (2,400 rad) popliteal LNC from mice primed into the hind footpads 8 d before with HEL or HEL peptides (1–14 nmol/mouse) emulsified in CFA. Culture conditions and assessment of IL-2 production were as described above. The MHC class II molecule involved in antigen presentation by LNC was determined by inhibition of T cell activation with the mAbs 12-2.16, anti-$I\text{-}A^{f,j,k,r,s,u}$ (20), and 14-4-4S, anti-I-E (21).

Chloroquine Treatment. Irradiated LNC were incubated in culture medium containing the lysosomotropic agent chloroquine (Sigma Chemical Co.) for 30 min at 37°C before adding antigen. After a 4-h incubation with chloroquine and antigen, the LNC were washed three times with RPMI, and their capacity to activate T cell hybridomas was tested as described above.

Anti-Thy-1 Cytotoxic Treatment. LNC (6×10^6/ml) were incubated with 1.5 μg/ml of purified anti-Thy-1.2 mAb (Becton Dickinson & Co., Mountain View, CA) for 1 h at 4°C. Cells were then washed once, resuspended in low-tox rabbit complement (Cedarlane Laboratories, London, Canada) at a final dilution of 1:15, incubated 45 min at 37°C, and then washed three times. T cell depletion was assessed by lack of responsiveness to Con A (Sigma Chemical Co.) and by cytofluorimetric analysis with biotinylated anti-CD4 mAb (Becton Dickinson & Co.).

Results

Activation of Class II–restricted, HEL Peptide–specific T Cell Hybridomas by Irradiated LNC from HEL-primed Mice. The basic experimental design of the present study relied on the hypothesis that draining LNC from HEL-primed mice would include APC expressing on their surface immunologically detectable complexes of naturally processed HEL peptides bound to class II MHC molecules. To test this hypothesis, C3H and DBA/2 mice were immunized with HEL-CFA. 8 d later, draining LNC were irradiated and incubated, in the absence of added antigen, with the T cell hybridoma 3A9, recognizing the HEL peptide 46–61 bound to $I\text{-}A^k$ molecules (19), or with the T cell hybridoma 1H11.3, recognizing the HEL peptide 108–116 bound to $I\text{-}E^d$ molecules (8, 22). Results in Fig. 1 demonstrate that LNC from HEL-primed C3H mice induce IL-2 production by 3A9 but not by 1H11.3 T cells. Conversely, LNC from HEL-primed DBA/2 mice induce IL-2 production by 1H11.3 but not by 3A9 T cells. Activation of T cell hybridomas is maximal using as APC $0.5\text{–}1 \times 10^6$ LNC/well from HEL-primed mice. LNC from CFA-primed mice of either strain fail to induce activation

Table 1. *T Cell Hybridomas Used in the Present Study*

Denomination	HEL peptide specificity	Amino acid sequence	Restriction	Responsiveness HEL	Responsiveness peptide	Reference
				μM		
1C5.1	46–61	NTDGSTDYGILQINSR	A^k	0.3	0.1	This paper
3A9	46–61		A^k	0.02	0.04	19
2D4.1	112–129	RNRCKGTDVQAWIRGARL	A^k	0.3	0.03	This paper
1H11.3	108–116	WVAWRNRCK	E^d	0.5	0.3	8
2G7.1	1–18	KVFGRCELAAAMKRHGLD	E^k	0.3	0.3	18

The responsiveness of T cell hybridomas was estimated by titrating antigen on LK-35.2 cells as APC. Values (mean of three to four experiments) represent the amount of HEL or peptide inducing 50% of the maximal response. HEL peptide sequences are indicated by the single-letter code.

of HEL-specific T cell hybridomas. Similarly, irradiated LNC from HEL-primed mice, cultured alone, fail to produce IL-2 (not shown). A direct relationship exists between the dose of antigen injected and the degree of T cell hybridoma activation induced by LNC from primed mice. This is exemplified in Fig. 2 by the response of hybridoma 1C5.1, recognizing the HEL peptide 46–61 together with I-A^k molecules.

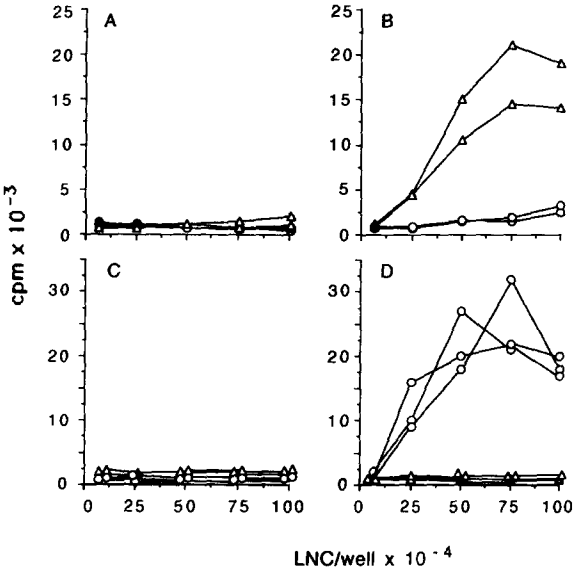

Figure 1. Activation of MHC class II-restricted T cell hybridomas by irradiated lymph node cells from HEL-primed mice. C3H, H-2^k (*A* and *B*) or DBA/2, H-2^d (*C* and *D*) mice were immunized into the hind footpads with CFA alone (*A* and *C*) or with 7 nmol/mouse HEL-CFA (*B* and *D*). 8 d later popliteal LNC from individual mice were irradiated (2,400 rad) and the indicated cell numbers cultured with 5 × 10^4 cells/well of T hybridomas 3A9 (Δ, I-A^k/HEL 46–61) or 1H11.3 (O, I-E^d/HEL 108–116). After 24 h, antigen-specific T cell growth factor production was determined by adding 50-μl aliquots of culture supernatant to 10^4 CTLL cells for an additional 24 h. [^{3}H]Thymidine (1 μCi/well) was added during the last 5 h of culture. Data are presented as mean thymidine incorporation (cpm) from triplicate cultures. Background proliferation of CTLL cells was 600 cpm.

Draining LNC from C3H mice primed with different doses of HEL (Fig. 2, *A–C*) or of HEL peptide 46–61 (Fig. 2, *D–F*) induce a similar, dose-dependent, activation of hybridoma 1C5.1.

These results demonstrate that lymph node cells from HEL-primed mice contain APC expressing complexes of HEL peptides and class II MHC molecules, as detected by appropriate T cell hybridomas. The antigen-presenting activity can be detected in LNC 6–12 d after immunization, but it is maximal after 8 d (not shown).

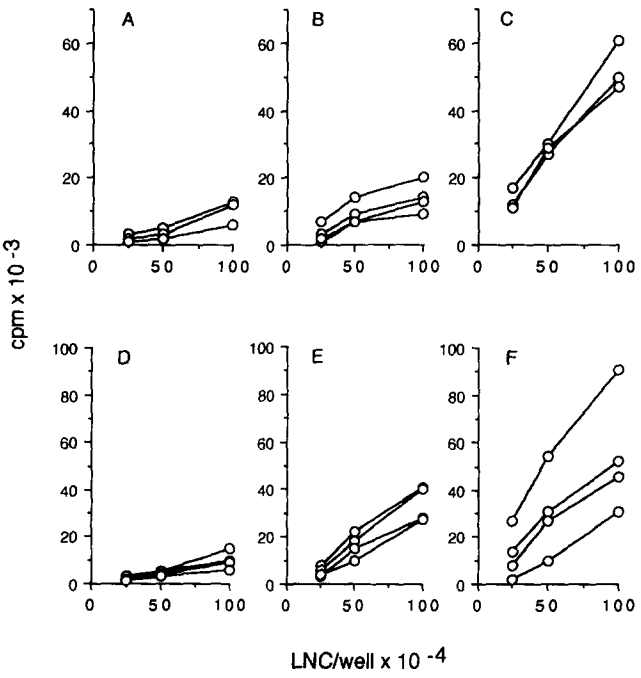

Figure 2. Direct relationship between the dose of antigen injected and the degree of T cell hybridoma activation. C3H mice were immunized with 1 nmol (*A*), 3 nmol (*B*), or 10 nmol/mouse (*C*) HEL in CFA, or with 1 nmol (*D*), 3 nmol. (*E*), or 10 nmol/mouse (*F*) HEL peptide 46–61 in CFA. Culture conditions were as in Fig. 1, except that the read-out T cell hybridoma was 1C5.1 (I-A^k/HEL 46–61). Data from individual mice are presented as in Fig. 1.

Antigenic Complexes between HEL-derived Peptides and Class II Molecules Are Formed In Vivo. To assess whether antigenic complexes are formed in vivo, rather than by carry-over of HEL in vitro, we incubated LNC from HEL-primed mice with the lysosomotropic agent chloroquine before addition of the T cell hybridoma 1C5.1. Results in Fig. 3 demonstrate that chloroquine fails to interfere with antigen presentation by LNC from HEL-primed mice, whereas it prevents presentation of HEL but not of the HEL peptide 46–61 by LNC from CFA-primed mice. These results indicate the presence of preformed complexes between antigenic HEL peptides and class II molecules in LNC from HEL-primed mice.

To confirm the MHC class II restriction of LNC antigen-presenting activity, anti-I-A and anti-I-E mAbs were added to cultures of LNC from HEL-primed mice and T cell hybridomas. Results in Fig. 4 *A* demonstrate that anti-I-A, but not anti-I-E, antibodies abrogate antigen presentation to the I-A^k-restricted hybridoma 1C5.1. Conversely, anti-I-E, but not anti-I-A, antibodies completely inhibit activation of the I-E^d-restricted hybridoma 1H11.3 (Fig. 4 *B*).

MHC-specific Inhibition of I-A^k-restricted T Cell Proliferation by Administration of I-A^k-binding Peptide Competitors. The mouse lysozyme (ML) peptide 46–62 binds strongly to I-A^k molecules, but it fails to bind to I-E^k or I-E^d molecules (5, 10, 23). Previous experiments have demonstrated that injection of this peptide competitor inhibits in vivo T cell activation by I-A^k-binding antigenic peptides (10). The exquisite specificity of this inhibitory activity is illustrated in Fig. 5. The T cell response induced by HEL in C3H mice can be recalled by several dominant HEL peptides (11, 24), among them 1–18 and 112–129 (Fig. 5 *A*). Since the response to HEL 1–18 is I-E^k restricted and the response to 112–129 I-A^k restricted (11), we asked whether coinjection of HEL and ML 46–62 in C3H mice would inhibit only I-A^k-restricted T cell induction. Results in Fig. 5 *B* demonstrate that injection of ML 46–62 completely inhibits T cell proliferation to HEL 112–129, but, in the same lymph node cell population, there is no inhibition of the T cell response to HEL 1–18. ML 46–62 is nonimmunogenic in mice and therefore the observed inhibition cannot be ascribed to induction of clonal dominance

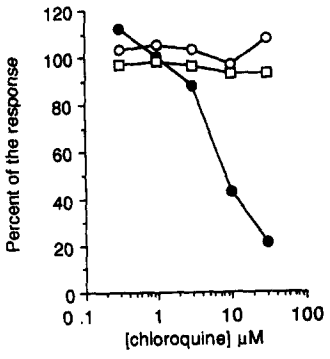

Figure 3. Antigenic complexes between HEL-derived peptides and class II molecules are formed in vivo. Irradiated LNC from C3H mice primed with 14 nmol/mouse HEL in CFA (10^6 cells/well, ○) or with CFA only (2.5×10^5 cells/well, □ and ●) were incubated with the indicated concentrations of the lysosomotropic agent chloroquine 30 min before the addition of 2 μM HEL (●) or 2 μM HEL 46–61 (□). After a 4-h incubation at 37°C, the LNC were washed, the T cell hybridoma 1C5.1 was added, and the experiment continued as in Fig. 1. Control responses were 165,482 (○), 177,128 (●), and 321,537 (□) cpm. Background proliferation of CTLL cells was 566 cpm.

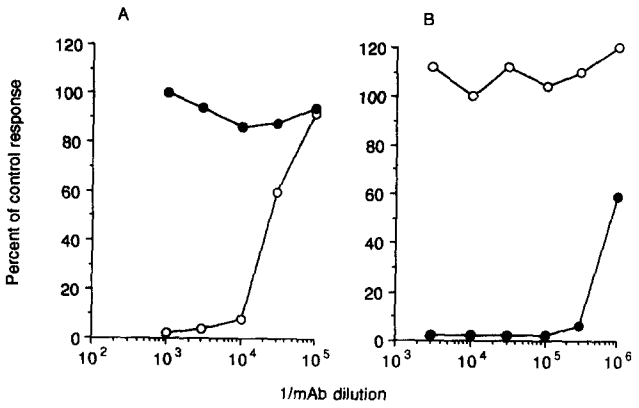

Figure 4. The antigen-presenting activity of LNC from HEL-primed mice is MHC class II restricted. Irradiated LNC from C3H (*A*) or B10.D2, H-2^d (*B*) mice primed with 14 nmol/mouse HEL in CFA were cultured (0.5×10^6 cells/well) in medium containing the indicated dilutions of mAb 10-2.16, anti-I-A (○), or 14-4-4S, anti-I-E (●), and the T cell hybridomas 1C5.1 (A^k/HEL 46–61, *A*) or 1H11.13 (E^d/HEL 108–116, *B*). IL-2 production was measured as in Fig. 1. Control responses were 79,582 (*A*) and 15,208 (*B*) cpm. Background proliferation of CTLL cells was 354 cpm.

by the competitor peptide. However, in the experiment illustrated in Fig. 5, mice were injected with HEL and inhibition of T cell proliferation to 112–129 could have been mediated, across an antigen bridge, by suppressor T cells induced by ML 46–62 (25). To eliminate this possibility, we used as antigen the HEL peptide 112–129 and as competitor the sequence 46–61 either of hen or mouse origin. Results in Fig. 6 *A* demonstrate that either HEL 46–61 or ML 46–62, coinjected with HEL 112–129, inhibit T cell proliferation to 112–129. The degree of inhibition is unrelated to the immunogenicity of the inhibitory peptide, since a similar inhibition is exerted by the immunogenic peptide HEL 46–61 and the nonimmunogenic ML 46–62. The inhibition of T

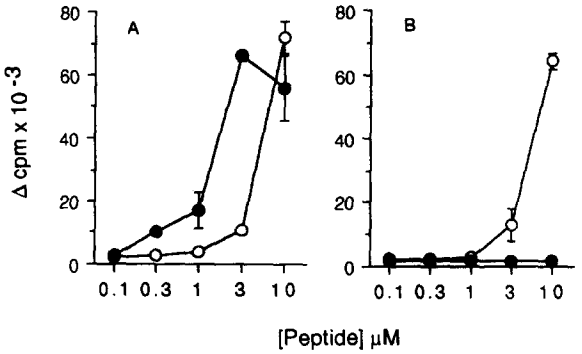

Figure 5. MHC-specific inhibition of T cell priming. C3H mice were immunized subcutaneously into the hind footpads with 14 nmol/mouse HEL in CFA (*A*) or with a mixture of 14 nmol HEL and 140 nmol ML 46–62/mouse (*B*). 8 d later the popliteal LNC from four mice were pooled and restimulated in vitro with the indicated concentrations of the HEL peptides 1–18 (○) or 112–129 (●). After 3 d of culture, 1 μCi [^{3}H]thymidine was added and the cells harvested 10 h later. Data are presented as mean ± SEM of cpm from triplicate cultures. Background (no antigen) cpm were 2,234 (*A*) and 1,244 (*B*).

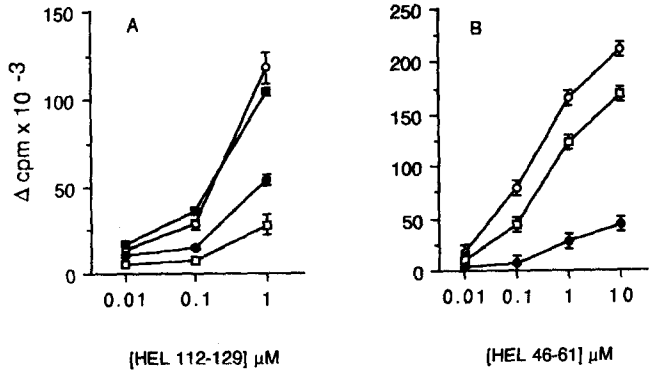

Figure 6. Reciprocal inhibition of T cell priming by injection of peptide competitors non homologous to the antigenic peptides. C3H mice (*A*) were immunized with 14 nmol/mouse HEL 112–129 alone (○) or mixed either with 100 nmol ML 46–62 (□), HEL 46–61 (●), or HEL 64–77 (■). Alternatively (*B*), C3H mice were immunized with 1 nmol/mouse HEL 46–61 alone (○) or mixed either with 100 nmol 112–129 (●) or 100 nmol 64–77 (□). 8 d later popliteal LNC from four mice were pooled and restimulated in vitro with different concentrations of the indicated peptides. Results are expressed as in Fig. 5. Background values ranged from 2,799 to 10,236 cpm. The amino acid sequence, in the single-letter code, of the HEL peptide 64–77 is CNDGRTPGSRNLCN, and that of ML 46–62 DRGDQSTDYGIFQINSR.

cell activation in vivo depends on the molar ratio between antigenic and competitor peptide, since HEL 46–61 can also function as antigen and HEL 112–129 as competitor (Fig. 6 *B*). In either case, no inhibition of T cell activation is observed by coinjection of HEL 64–77, which does not bind to I-A^k (11).

MHC-specific Inhibition of I-A^k-restricted Antigen Presentation by Administration of I-A^k-binding Peptide Competitors. Having established that detectable antigenic complexes are formed in vivo between class II MHC molecules and peptides derived from HEL processing, we examined the effect of administering a class II-binding peptide competitor on the in vivo formation of antigenic complexes. C3H mice were immunized with HEL and 64–77 in CFA, or with an emulsion containing HEL and 112–129. Alternatively, HEL 46–61 replaced HEL as antigen. 8 d later, irradiated LNC were tested for their capacity to activate the I-A^k-restricted hybridoma 1C5.1, specific for HEL 46–61. Results in Fig. 7 demonstrate that, in either case, coinjection of the I-A^k-binding peptide competitor inhibits profoundly the I-A^k-restricted antigen-presenting activity. The specificity of the inhibitory effect is also indicated by the unimpaired ability of LN APC from competitor-injected mice to process and present in vitro exogenous HEL. This demonstrates that APC from mice injected with a class II-blocking peptide able to prevent the formation of antigenic complexes in vivo can present exogenous antigens added in vitro, presumably due to newly synthetized MHC class II molecules.

To correlate the inhibition of T cell proliferation with the inhibition of antigen presentation, C3H mice were injected with HEL alone, or mixed with either HEL 64–77 or 112–129. 8 d later, antigen presentation to the 46–61-specific T cell hybridoma 1C5.1 and T cell proliferation to the HEL peptide 46–61 was simultaneously tested in the same LNC populations. Results in Fig. 8 demonstrate that injection of the I-A^k-binding peptide HEL 112–129 inhibits equally well I-A^k-restricted antigen presentation and T cell proliferation by LNC. This indicates that MHC blockade is equally effective in inhibiting antigen presentation to T cell hybridomas as well as to bulk T cells.

Inhibition of Antigen-presenting Activity Does Not Require T Cells. To exclude the possibility that suppressor T cells induced by HEL 112–129 in the LNC population could inhibit activation of T cell hybridomas, T cell–depleted LNC from HEL 46–61-primed C3H mice, coinjected with HEL 112–129 or with HEL 64–77, were used as APC. Results in Fig. 9

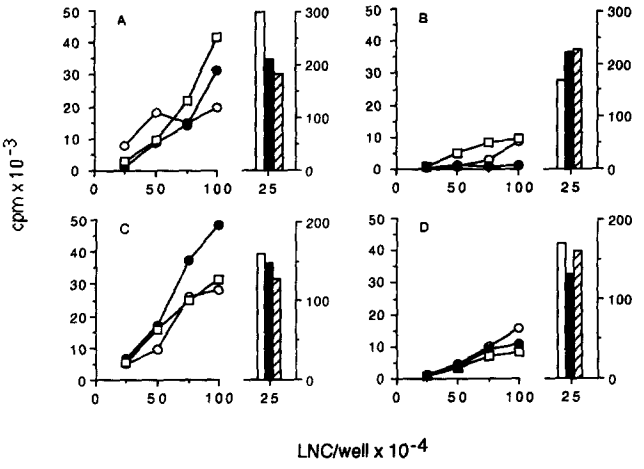

Figure 7. MHC-specific inhibition of antigen presentation. C3H mice were immunized with 1 nmol HEL/mouse mixed either with 100 nmol HEL 64–77 (*A*) or 100 nmol 112–129 (*B*), or with 3 nmol HEL 46–61/mouse mixed either with 100 nmol HEL 64–77 (*C*) or 100 nmol 112–129 (*D*). Irradiated LNC from individual mice were cultured with the T cell hybridoma 1C5.1 (A^k/HEL 46–61), and Il-2 production was measured as in Fig. 1. Bars represent IL-2 production induced by 2.5 × 10^5 LNC/well from the same individual mice and 2 μM HEL in 1C5.1 cells. Background proliferation of CTLL cells was 410 (*A* and *B*) and 254 (*C* and *D*) cpm.

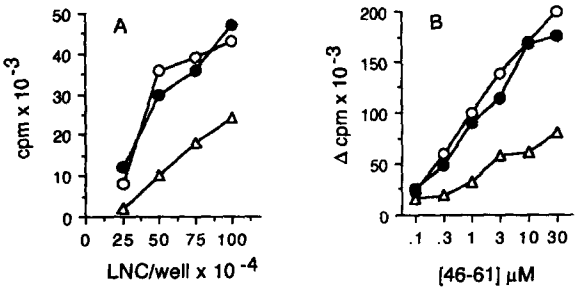

Figure 8. Correlation between inhibition of antigen presentation and inhibition of T cell priming. C3H mice were immunized with 3 nmol HEL/mouse alone (○) or mixed either with 100 nmol HEL 64–77 (●) or 100 nmol 112–129 (Δ). 8 d later, LNC from four mice were pooled, and either irradiated and cultured with the T cell hybridoma 1C5.1 (*A*) or restimulated in vitro with the indicated concentrations of HEL 46–61 (*B*). Results in *A* and *B* are expressed as in Figs. 1 and 5, respectively.

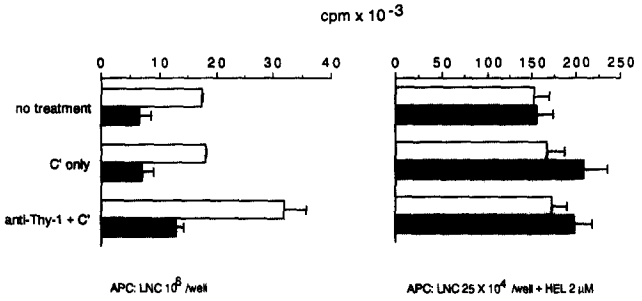

Figure 9. Inhibition of antigen-presenting activity does not require T cells. C3H mice were immunized with 1 nmol HEL 46–61/mouse mixed either with 100 nmol HEL 64–77 (□) or 100 nmol 112–129 (■). 8 d later, LNC from four mice were pooled, treated with anti-Thy-1 mAb plus C', irradiated, and cultured with the T cell hybridoma 1C5.1. Cultures were set up with 10^6 LNC/well (*left*) or with 2.5×10^5 LNC/well and 2 μM HEL (*right*), and the experiment continued as in Fig. 1. Data are shown as mean ± SEM cpm from triplicate cultures. Background proliferation of CTLL cells was 332 cpm.

demonstrate that the antigen-presenting capacity of T cell–depleted LNC from 46–61-primed mice is not impaired, indicating that antigen presentation is not mediated by T cells. A similar inhibition of T hybridoma activation is observed in untreated or T cell–depleted LNC from mice coinjected with 112–129, indicating that inhibition of T hybridoma activation does not depend on lymph node T cells. Comparable results are obtained using HEL as antigen (not shown). T cell depletion was complete, as assessed by Con A responsiveness and cytofluorimetric analysis, with anti-CD4 mAb (data not shown). Untreated or T cell–depleted LNC from control or competitor-injected mice could present equally well in vitro exogenous HEL to the T cell hybridoma 1C5.1. Therefore, these results rule out inhibition of T hybridoma activation by suppressor T cells.

Detection of Competitor/I-A^k Complexes Correlates with Inhibition of I-A^k-restricted Antigen Presentation. Next, we wished to demonstrate that inhibition of antigen presentation is associated with the presence of the competitor peptide in the binding site of class II molecules. LNC from C3H mice, injected with HEL 46–61 alone or coinjected with either the I-E^k-binding peptide 1–18 or with 112–129, were used to activate the specific T cell hybridomas. As expected, a strong decrease in the I-A^k-restricted antigen-presenting activity of LNC was only observed in mice injected with the I-A^k-binding competitor HEL 112–129, whereas no effect was induced by injection of the I-E^k-binding peptide HEL 1–18 (Fig. 10 *A*). Both peptides are bound to the appropriate class II molecules, as detected by activation of specific T cell hybridomas (Fig. 10, *B* and *C*). These results demonstrate that inhibition of the I-A^k-restricted antigen presentation by LNC from competitor-injected mice correlates with the presence of the competitor peptide in the binding site of I-A^k molecules.

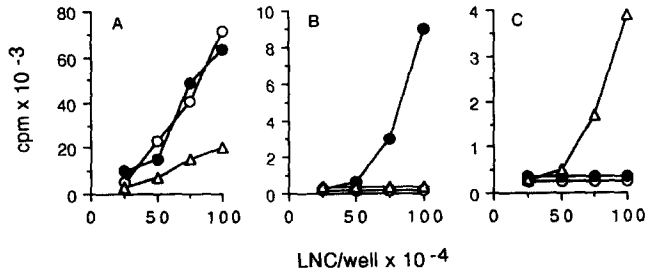

Figure 10. Detection of competitor/I-A^k complexes correlates with inhibition of I-A^k-restricted antigen presentation. C3H mice were immunized with 3 nmol HEL 46–61/mouse (○) alone or mixed either with 100 nmol HEL 1–18 (●) or with 100 nmol HEL 112–129 (△). 8 d later, LNC from four mice were pooled, treated with anti-Thy-1 mAb plus C', irradiated, and then cultured with the T cell hybridomas 1C5.1 (*A*), 2G7.1 (*B*), or 2D4.1 (*C*). Results are expressed as in Fig. 1. Background proliferation of CTLL cells was 230 cpm.

Discussion

In vivo administration of class II–binding competitor peptides can selectively inhibit the activation of T cells induced by antigenic peptides binding to the same class II molecule (10, 14, 23). Since the inhibition of T cell priming is specific for the class II molecule binding the competitor peptide, and it does not depend on homology between competitor and antigenic peptides, it was inferred, although not directly proven, that the underlying in vivo mechanism was inhibition of antigen presentation by competitive binding to the class II molecule restricting the T cell response. Data in the present paper now provide evidence for this mechanism.

Inhibition of T cell activation by injection of class II–binding peptides could have several possible explanations, apart from competition for antigen presentation. If the inhibitor peptide is structurally closely related to the antigenic peptide, antigen-specific rather than MHC-specific mechanisms may be involved. These include induction of T cell tolerance (26), induction of suppressor T cells (27), or production by T cells of different lymphokines (28). If inhibitory and antigenic peptides are nonhomologous, but the inhibitory peptide is immunogenic, inhibition of the T cell response to antigen could result from clonal dominance induced by the inhibitory peptide.

Our previous experiments have utilized as inhibitor a nonimmunogenic class II–binding peptide nonhomologous to the antigen, suggesting MHC blockade as the cause for inhibition of T cell activation (10, 23). Although, in this case, the mechanism responsible for inhibition of T cell priming appears to be MHC blockade, it remains to be seen whether this applies to all situations in which T cell activation has been prevented by injection of class II–binding peptides, and particularly in the case of competitors sharing extensive sequence homology with the antigenic peptide. In fact, in the latter situation, the inhibition of T cell activation could be easily accounted for by stimulation of suppressor T cells or by induction of T cell tolerance.

In mice of H-2^u haplotype, the acetylated NH_2-terminal peptide (amino acid residues 1–9) of myelin basic protein is able to induce encephalitogenic T cells (29). Using this experimental model, several authors have demonstrated that coinjection of competitor and encephalitogenic peptides can prevent the clinical development of EAE. Disease prevention was

related to inhibition of encephalitogenic peptide binding to I-A^u molecules (12, 13), with the consequent failure to activate encephalitogenic T cells. The evidence for this mechanism was based on the observation that peptides able to prevent EAE induction were binding to I-A^u better than encephalitogenic peptides, both in assays using purified class II molecules (12) and in competition for antigen presentation in vitro (13). However, since inhibitor and encephalitogenic peptides were highly homologous, induction of T cell tolerance or of suppressor T cells able to prevent disease induction could not be excluded (16, 17).

EAE can also be induced in H-2^s mice by a peptide derived from the murine proteolipid protein (PLP 139–151), and coadministration of this encephalitogenic peptide together with an unrelated peptide binding to I-A^s can prevent EAE induction (14). Since in this study encephalitogenic and competitor peptides did not share sequence homology, in this case, induction of T cell anergy or suppression by the competitor peptide does not appear to be the mechanism of disease prevention, and indeed this is most likely accounted for by MHC blockade. However, the inhibitory peptides used were themselves immunogenic, leaving open the possibility for a mechanism of clonal dominance in preventing induction of encephalitogenic T cells.

To obtain evidence for MHC blockade in vivo, we first established an ex vivo experimental model to detect complexes of naturally processed antigenic peptides generated in vivo and expressed by MHC class II molecules on the surface of APC. This was accomplished by immunizing mice with HEL in CFA and assessing the expression of antigenic complexes in LNC draining the injection site by their ability to activate HEL peptide–specific, class II–restricted T cell hybridomas. Only T hybridomas responding in vitro to low antigen concentrations could be activated by in vivo formed antigenic complexes. Activation of T cell hybridomas like 1H11.3 only requires about 50 antigenic complexes (30), suggesting that in vivo processing of HEL may give rise to a relatively low number of complexes between antigenic HEL peptides and class II molecules.

Injection of I-A^k-binding peptides together with antigen in mice of H-2^k haplotype prevents the formation in vivo of antigen-I-A^k complexes, as assessed by activation of HEL-specific T cell hybridomas. Inhibition of T hybridoma activation is observed using as APC irradiated, T cell–depleted LNC from mice coinjected with antigenic and competitor peptides, ruling out a possible involvement of specific or nonspecific suppressor T cells in preventing T cell activation. Since, in the experiments described here, the I-A^k-binding peptides used as competitors were always nonhomologous to the antigenic peptides, tolerance or T cell anergy as possible mechanisms inhibiting T cell activation are also excluded.

Besides excluding possible alternative mechanisms, results in this paper directly support MHC blockade as the mechanism preventing T cell activation in mice injected with a class II–binding peptide competitor. In mice coinjected with HEL 46–61 and a molar excess of HEL 112–129, both binding to I-A^k molecules, competitive inhibition takes place in vivo between these two peptides for the formation of peptide/I-A^k complexes. Under these conditions, inhibition of class II–restricted T cell induction by administration of MHC class II binding peptide competitors is associated with in vivo inhibition of antigen presentation and with occupancy by the competitor of class II binding sites. It is important to note that in vivo MHC blockade is equally effective in inhibiting antigen presentation to T cell hybridomas and to bulk T cells, indicating the relevance of this immunosuppressive mechanism.

In conclusion, we have demonstrated that injection of a class II–binding peptide competitor can selectively impair antigen presentation to class II–restricted T cells. This effect appears to be due to MHC blockade, modulating T cell activation by preventing binding of antigenic peptides to class II molecules. It is hoped that this approach could be applicable to human systems to evaluate induction of selective immunosuppression in HLA-associated autoimmune diseases (31, 32).

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Address correspondence to Luciano Adorini, Preclinical Research 386-106, Sandoz Pharma Ltd., CH-4002 Basel, Switzerland. J.-C. Guéry is on leave of absence from INSERM U28, Hôpital Broussais, Paris, France.

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